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Review

T-cell epitope discovery technologies

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ABSTRACT

Despite tremendous potential utility in clinical medicine and research, the discovery and characterization of T-cell antigens has lagged behind most other areas of health research in joining the high-throughput '-omics' revolution. Partially responsible for this is the complex nature of the interactions between effector T cells and antigen-presenting cells. Further contributing to the challenge is the vastness of both the T-cell repertoire and the large number of potential T-cell epitopes. In this review, we trace the development of various discovery strategies, the technical platforms used to carry them out, and we assess the level of success achieved in the field today.

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1. Introduction

The elucidation of T-cell antigens is crucial to the understanding of the molecular etiology of immune related disorders and the development of novel therapeutic strategies. Reliable identification of T-cell antigens would, in particular, address an unmet need in the fields of cancer immunology [1], autoimmunity [2] and infectious disease [3]. T-cell epitopes are short peptides displayed at the surface of antigen-presenting cells by the membrane-bound major histocompatibility complex (MHC) proteins, which are categorized as either class I or II. Class I molecules are expressed on the surface of nearly every cell of the body and present a sampling of short (8–14 residue [4]) peptides derived from proteolytic turnover of proteins of both endogenous and exogenous origin. These MHC class I antigens are targets of direct attack from cytotoxic T-lymphocytes. MHC class II exists on the surface of professional antigen-presenting cells (pAPCs) and is responsible for priming naïve T-lymphocytes in peripheral lymphoid tissue. Notably,

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peptide determinants presented on the surface of class II molecules are not subject to strict length constraints like their MHC class I counterparts. T-cell recognition of peptide-MHC (pMHC) is mediated by the αβ-T-cell receptor (TCR), a heterodimeric integral T-cell membrane protein composed of an α and β subunit, each encoded at a separate genomic locus. Each of these TCR subunit genes has a hypervariable region that encodes complementarity-determining region 3 (CDR3), which is the primary region of direct engagement with MHC-presented peptide epitopes. This hypervariability is derived from stochastic somatic rearrangement of gene segments present within the germline locus of each subunit [5]. Post-recombination, nascent T cells undergo positive and negative selection against self pMHC to yield a diverse repertoire optimized for tolerance of self antigens but poised for recognition of any foreign antigen that may be encountered.

The discovery of T-cell epitopes has proven, historically, to be a difficult endeavor given numerous characteristics of T-cell antigen recognition that must be accounted for. Firstly, the extreme diversity of the T-cell repertoire generally ensures that clonotypes of interest are present in very low numbers. Moreover, T-cell epitope recognition is a notoriously low affinity interaction that must occur in the context of polygenic and highly polyallelic MHC molecules. Meanwhile, processing and presentation of both exogenous and endogenous peptides on MHC molecules makes for an enormous T-cell epitope space to be screened. Contributing to the complexity associated with T-cell antigen discovery is the substantial level of cross-reactivity present in the T-cell repertoire. Theoretical calculations have estimated the number of pMHC antigens recognized by a single TCR to be on the order of 10^6 [6] and such estimates have since received experimental support [7]. Further, it has been noted that these cross-reactive epitopes need not share significant sequence similarity and that TCRs are capable of binding different pMHC via numerous different mechanisms [8]. With respect to antigen-discovery applications, these observations indicate the importance of shifting towards large-scale approaches such as combinatorial library screening to effectively probe pMHC/TCR reactivity. Importantly, from a practical standpoint, high-dimensional screening for cross-reactive epitopes can afford the opportunity for therapeutic intervention by revealing antigens with a higher capacity for priming a T-cell response than the natural epitopes restricted to the target pathology [9–11].

Extensive research and development efforts in the field of T-cell antigen discovery have been ongoing over the previous three decades (summarized in Fig. 1) with many of these technologies poised for success in the new era of high-dimensional biological research. The subsequent sections of this review outline the evolution of T-cell antigen discovery and assesses some of the main challenges remaining.

2. Early genomic/cDNA library screening

Initial TCR antigen discovery efforts were focused on melanoma since these tumor cells are generally more amenable to the creation of stable cell-lines [12] and are highly mutated, thus providing an abundance of mutational epitopes to characterize. In the late 1980s and early 1990s, pioneering work was done in which cytotoxic T-lymphocyte (CTL)-sensitive cells isolated from tumor were selected by co-culture with reactive CTLs until stable antigen-loss variants were isolated. A cosmid library could then be constructed from the genomic DNA of the original cells, transfected into the antigen-loss variant line, and co-cultured with T-cell clones of interest derived from patient peripheral blood. Subsequent chromium-51 release assays would reveal transfectants in which CTL sensitivity was restored and cosmid vectors could be recovered for characterization [13]. These experiments led to the discovery of the now well-known melanoma associated antigens, MAGE-1, -2, and -3. In another study, a similar approach was used whereby melanoma derived cDNA libraries instead of genomic DNA libraries were transfected into a non-melanoma cell line for screening against patient derived tumor-infiltrating lymphocytes (TIL) [14]. These investigations led to the identification of another classic melanoma antigen, MART-1.

A major impediment of the above methodologies was the requirement to create stable target cell lines expressing genes from the tissue under interrogation. Many other important diseases,

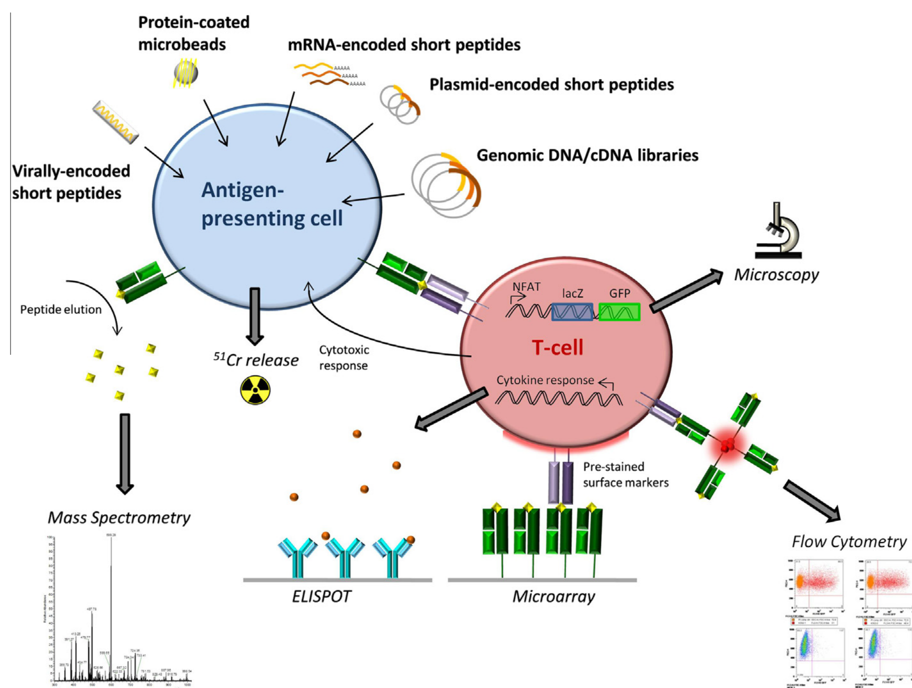

Fig. 1. A summary of the various T-cell antigen discovery approaches with respect to both antigen-presentation strategies and assay methods.

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